REMARKS

Applicants respectfully request reconsideration of the claims in view of the following remarks. Claims 80-85, 94-100, 104, and 106-108 are currently pending.

35 U.S.C. § 102

Claims 80-84, 94-99, and 104 were rejected under 35 U.S.C. § 102(e) as anticipated by U.S. Patent No. 6,485,938 (hereinafter the '938 patent). Applicants respectfully traverse this rejection.

The present application claims priority to a number of patent applications including provisional application 60/145,698 (hereinafter the '698 application), filed on July 26, 1999. The filing date of at least the '698 application (July 26, 1999) predates the earliest priority date of the '938 patent.

The Office Action alleges "an antibody or antibody fragment thereof that specifically binds residues 20-105 of SEQ ID NO:2 and inhibits EG-VEGF induced proliferation of endothelial cells" as set forth in claim 80 is not disclosed in the '698 application. The Office Action also alleges the specific residues "20-105" of SEQ ID NO:2, to which the claimed antibody or antibody fragment binds, is not disclosed in the '698 application. Applicants respectfully disagree.

EG-VEGF (SEQ ID NO:2) is referred to as PRO1186 (SEQ ID NO:165; Figure 66) in the '698 application. The '698 application discloses that the term "PRO1186" or "PRO1186 polypeptide" encompasses native-sequence PRO1186 polypeptide variants. See, for example, page 44, line 29 to page 45, line 20. The native-sequence PRO1186 polypeptide can be a mature or full-length native sequence PRO1186 comprising the amino acid sequence of Figure 66 (SEQ ID NO:165). See, for example, page 46, line 19 to page 47, line 7.

Fragments of the native polypeptide include, but are not limited to, polypeptide variants from which the native N-terminal signal sequence has been fully or partially deleted. See, for example, page 47, lines 8-10. Analysis of the full-length PRO1186 sequence indicated that amino acids 1-19 of PRO1186 (SEQ ID NO:165) comprise a signal sequence. See, for example, page 279, lines 4-5. In addition, the '698 application defines a "PRO1186 polypeptide variant" as an active PRO1186 polypeptide having at least about 80% amino acid sequence identity with

residues 20 to 105 of the PRO1186 polypeptide shown in Figure 66 (SEQ ID NO:165). See, for example, page 55, lines 21-24 and page 77, lines 14-29.

The 698' application discloses inhibiting the mitogenic or angiogenic activity of PRO1186 polypeptides with an antagonist. See, for example, page 126, lines 8-14. Antagonist antibodies that bind PRO1186 are disclosed, for example, at page 19, lines 3-21, at page 126, line 27 to page 127, line 2, and at pages 208-215. As discussed above, PRO1186 is defined in the '698 application as encompassing full-length, mature, or variant PRO1186 polypeptides, including the mature form comprising residues 20-105 of SEQ ID NO:165. Example 38 in the '698 application describes an assay for determining if a molecule has cell proliferation activity. The specification demonstrates that PRO1186 induces proliferation of ACE cells (pages 280-281) and discloses methods for identifying an antibody that inhibits the cell proliferation activity of PRO1186. See, for example, page 166, lines 6-19.

The above referenced pages and figures from the '698 application are attached for the Examiner's convenience.

In view of the forgoing, Applicants submit the '938 patent does not anticipate any of the claims. The filing date of the '698 application (July 26, 1999) predates the earliest priority date of the '938 patent. The language of the claims is consistent with the description of EG-VEGF in the '698 application and the specification. Antibodies and antibody fragments that bind EG-VEGF polypeptides having at least 80% amino acid sequence identity with the amino acid sequence of residues 20-105 of SEQ ID NO:2 are clearly described in both the specification and the '698 Application. Accordingly, withdrawal of the rejection is respectfully requested.

Non-Statutory Obviousness-Type Double Patenting

Claims 80-85, 100, and 106-108 were provisionally rejected on the grounds of non-statutory obviousness-type double patenting as being unpatentable over claims 16-17 of copending Application No. 10/205,654. Applicants acknowledge the rejection and request that the rejection be held in abeyance until allowable subject matter is indicated.

If a provisional non-statutory obviousness-type double patenting (ODP) rejection is the only rejection remaining in the earlier filed of the two pending applications and the later-filed application is rejectable on other grounds, the Examiner should withdraw the provisional ODP

rejection and permit the earlier filed application to issue as a patent without a terminal disclaimer. MPEP § 804(I)(B)(1). Applicants note that the present application was filed on December 19, 2001, while copending Application No. 10/205,654 was filed on November 26, 2002. The present application is therefore the earlier filed of the two applications.

Conclusion

In view of the above remarks, Applicants respectfully request a Notice of Allowance. If the Examiner believes a telephone conference would advance the prosecution of this application, the Examiner is invited to telephone the undersigned at the below-listed telephone number.

Respectfully submitted,

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Date: October 3, 2006

Eric E. DeMaster
Reg. No. 55,107

23552
PATENT TRADEMARK OFFICE

FIGURE 66

Signal peptide: Amino acids 1-19

MRGATRVSIMLLLVTVSDCAVITGACERDVQCGAGTCCAISLWLRGLRMCTPLGREGEECHP
GSHKVPFFRKRKHHTCPCLPNLLCSRFPDGRYRCSMDLKNINF

PRO366, PRO535, PRO819, PRO826, PRO1160, PRO1186 or PRO1246 polypeptide which may optionally be identified by the methods described above.

One type of antagonist of a PRO172, PRO175, PRO178, PRO188, PRO356, PRO179, PRO197, PRO198, PRO182, PRO195, PRO200, PRO211, PRO217, PRO219, PRO221, PRO224, PRO228, PRO245, PRO246, PRO258, PRO261, PRO272, PRO301, PRO322, PRO328, PRO331, PRO364, PRO366, PRO535, PRO819, PRO826, PRO1160, PRO1186 or PRO1246 polypeptide that inhibit one or more of the functions or activities of the PRO172, PRO175, PRO178, PRO188, PRO356, PRO179, PRO197, PRO198, PRO182, PRO195, PRO200, PRO211, PRO217, PRO219, PRO221, PRO224, PRO228, PRO245, PRO246, PRO258, PRO261, PRO272, PRO301, PRO322, PRO328, PRO331, PRO364, PRO366, PRO535, PRO819, PRO826, PRO1160, PRO1186 or PRO1246 polypeptide is an antibody. Hence, in another aspect, the invention provides an isolated antibody that binds a PRO172, PRO175, PRO178, PRO188, PRO356, PRO179, PRO197, PRO198, PRO182, PRO195, PRO200, PRO211, PRO217, PRO219, PRO221, PRO224, PRO228, PRO245, PRO246, PRO258, PRO261, PRO272, PRO301, PRO322, PRO328, PRO331, PRO364, PRO366, PRO535, PRO819, PRO826, PRO1160, PRO1186 or PRO1246 polypeptide. In a preferred aspect, the antibody is a monoclonal antibody, which preferably has non-human complementarity-determining-region (CDR) residues and human framework-region (FR) residues. The antibody may be labeled and may be immobilized on a solid support. In a further aspect, the antibody is an antibody fragment, a single-chain antibody, or an anti-idiotypic antibody. Preferably, the antibody specifically binds to the polypeptide.

In a still further aspect, the present invention provides a method for diagnosing a disease or susceptibility to a disease which is related to a mutation in a PRO172, PRO175, PRO178, PRO188, PRO356, PRO179, PRO197, PRO198, PRO182, PRO195, PRO200, PRO211, PRO217, PRO219, PRO221, PRO224, PRO228, PRO245, PRO246, PRO258, PRO261, PRO272, PRO301, PRO322, PRO328, PRO331, PRO364, PRO366, PRO535, PRO819, PRO826, PRO1160, PRO1186 or PRO1246 polypeptide-encoding nucleic acid sequence comprising:

(a) isolating a nucleic acid sequence encoding a PRO172, PRO175, PRO178, PRO188, PRO356, PRO179, PRO197, PRO198, PRO182, PRO195, PRO200, PRO211, PRO217, PRO219, PRO221, PRO224, PRO228, PRO245, PRO246, PRO258, PRO261, PRO272,

additionally include cytotherapeutic agents such as cytotoxic agents, chemotherapeutic agents, growth-inhibitory agents, apoptotic agents, and other agents to treat cancer, such as anti-HER-2, anti-CD20, and other bioactive and organic chemical agents.

In a pharmacological sense, in the context of the present invention, a "therapeutically effective amount" of an active agent such as a PRO172, PRO175, PRO178, PRO188, PRO356, PRO179, PRO197, PRO198, PRO182, PRO195, PRO200, PRO211, PRO217, PRO219, PRO221, PRO224, PRO228, PRO245, PRO246, PRO258, PRO261, PRO272, PRO301, PRO322, PRO328, PRO331, PRO364, PRO366, PRO535, PRO819, PRO826, PRO1160, PRO1186 or PRO1246 polypeptide or agonist or antagonist thereto or an anti-PRO172, anti-PRO175, anti-PRO178, anti-PRO188, anti-PRO356, anti-PRO179, anti-PRO197, anti-PRO198, anti-PRO182, anti-PRO195, anti-PRO200, anti-PRO211, anti-PRO217, anti-PRO219, anti-PRO221, anti-PRO224, anti-PRO228, anti-PRO245, anti-PRO246, anti-PRO258, anti-PRO261, anti-PRO272, anti-PRO301, anti-PRO322, anti-PRO328, anti-PRO331, anti-PRO364, anti-PRO366, anti-PRO535, anti-PRO819, anti-PRO826, anti-PRO1160, anti-PRO1186 or anti-PRO1246 antibody, refers to an amount effective in the treatment of a cardiovascular, endothelial or angiogenic disorder in a mammal and can be determined empirically.

As used herein, an "effective amount" of an active agent such as a PRO172, PRO175, PRO178, PRO188, PRO356, PRO179, PRO197, PRO198, PRO182, PRO195, PRO200, PRO211, PRO217, PRO219, PRO221, PRO224, PRO228, PRO245, PRO246, PRO258, PRO261, PRO272, PRO301, PRO322, PRO328, PRO331, PRO364, PRO366, PRO535, PRO819, PRO826, PRO1160, PRO1186 or PRO1246 polypeptide or agonist or antagonist thereto or an anti-PRO172, anti-PRO175, anti-PRO178, anti-PRO188, anti-PRO356, anti-PRO179, anti-PRO197, anti-PRO198, anti-PRO182, anti-PRO195, anti-PRO200, anti-PRO211, anti-PRO217, anti-PRO219, anti-PRO221, anti-PRO224, anti-PRO228, anti-PRO245, anti-PRO246, anti-PRO258, anti-PRO261, anti-PRO272, anti-PRO301, anti-PRO322, anti-PRO328, anti-PRO331, anti-PRO364, anti-PRO366, anti-PRO535, anti-PRO819, anti-PRO826, anti-PRO1160, anti-PRO1186 or anti-PRO1246 antibody, refers to an amount effective for carrying out a stated purpose, wherein such amounts may be determined empirically for the desired effect.

As used herein, the terms "PRO172", "PRO175", "PRO178", "PRO188", "PRO356", "PRO179", "PRO197", "PRO198", "PRO182", "PRO195", "PRO200", "PRO211", "PRO217", "PRO219", "PRO221", "PRO224", "PRO228", "PRO245", "PRO246", "PRO258", "PRO261",

"PRO272", "PRO301", "PRO322", "PRO328", "PRO331", "PRO364", "PRO366", "PRO535", "PRO819", "PRO826", "PRO1160", "PRO1186", "PRO1246", "PRO172 polypeptide", "PRO175 polypeptide", "PRO178 polypeptide", "PRO188 polypeptide", "PRO356 polypeptide", "PRO179 polypeptide", "PRO197 polypeptide", "PRO198 polypeptide", "PRO182 polypeptide", "PRO195 polypeptide", "PRO200 polypeptide", "PRO211 polypeptide", "PRO217 polypeptide", "PRO219 polypeptide", "PRO221 polypeptide", "PRO224 polypeptide", "PRO228 polypeptide", "PRO245 polypeptide", "PRO246 polypeptide", "PRO258 polypeptide", "PRO261 polypeptide", "PRO272 polypeptide", "PRO301 polypeptide", "PRO322 polypeptide", "PRO328 polypeptide", "PRO331 polypeptide", "PRO364 polypeptide", "PRO366 polypeptide", "PRO535 polypeptide", "PRO819 polypeptide", "PRO826 polypeptide", "PRO1160 polypeptide", "PRO1186 polypeptide", or "PRO1246" polypeptide" when used herein encompass native-sequence PRO172, PRO175, PRO178, PRO188, PRO356, PRO179, PRO197, PRO198, PRO182, PRO195, PRO200, PRO211, PRO217, PRO219, PRO221, PRO224, PRO228, PRO245, PRO246, PRO258, PRO261, PRO272, PRO301, PRO322, PRO328, PRO331, PRO364, PRO366, PRO535, PRO819, PRO826, PRO1160, PRO1186 or PRO1246 polypeptides and PRO172, PRO175, PRO178, PRO188, PRO356, PRO179, PRO197, PRO198, PRO182, PRO195, PRO200, PRO211, PRO217, PRO219, PRO221, PRO224, PRO228, PRO245, PRO246, PRO258, PRO261, PRO272, PRO301, PRO322, PRO328, PRO331, PRO364, PRO366, PRO535, PRO819, PRO826, PRO1160, PRO1186 or PRO1246 polypeptide variants (which are further defined herein). The PRO172, PRO175, PRO178, PRO188, PRO356, PRO179, PRO197, PRO198, PRO182, PRO195, PRO200, PRO211, PRO217, PRO219, PRO221, PRO224, PRO228, PRO245, PRO246, PRO258, PRO261, PRO272, PRO301, PRO322, PRO328, PRO331, PRO364, PRO366, PRO535, PRO819, PRO826, PRO1160, PRO1186 or PRO1246 polypeptide may be isolated from a variety of sources, such as from human tissue types or from another source, or prepared by recombinant and/or synthetic methods.

A "native sequence" PRO172, PRO175, PRO178, PRO188, PRO356, PRO179, PRO197, PRO198, PRO182, PRO195, PRO200, PRO211, PRO217, PRO219, PRO221, PRO224, PRO228, PRO245, PRO246, PRO258, PRO261, PRO272, PRO301, PRO322, PRO328, PRO331, PRO364, PRO366, PRO535, PRO819, PRO826, PRO1160, PRO1186 or PRO1246 polypeptide comprises a polypeptide having the same amino acid sequence as a PRO172, PRO175, PRO178, PRO188, PRO356, PRO179, PRO197, PRO198, PRO182, PRO195,

PRO200, PRO211, PRO217, PRO219, PRO221, PRO224, PRO228, PRO245, PRO246, PRO258, PRO261, PRO272, PRO301, PRO322, PRO328, PRO331, PRO364, PRO366, PRO535, PRO819, PRO826, PRO1160, PRO1186 or PRO1246 polypeptide derived from nature. Such native sequence PRO172, PRO175, PRO178, PRO188, PRO356, PRO179, PRO197, PRO198, PRO182, PRO195, PRO200, PRO211, PRO217, PRO219, PRO221, PRO224, PRO228, PRO245, PRO246, PRO258, PRO261, PRO272, PRO301, PRO322, PRO328, PRO331, PRO364, PRO366, PRO535, PRO819, PRO826, PRO1160, PRO1186 or PRO1246 polypeptide can be isolated from nature or can be produced by recombinant and/or synthetic means. The term "native sequence" PRO172, PRO175, PRO178, PRO188, PRO356, PRO179, PRO197, PRO198, PRO182, PRO195, PRO200, PRO211, PRO217, PRO219, PRO221, PRO224, PRO228, PRO245, PRO246, PRO258, PRO261, PRO272, PRO301, PRO322, PRO328, PRO331, PRO364, PRO366, PRO535, PRO819, PRO826, PRO1160, PRO1186 or PRO1246 polypeptide specifically encompasses naturally-occurring truncated or secreted forms (*e.g.*, an extracellular domain sequence), naturally-occurring variant forms (*e.g.*, alternatively spliced forms), and naturally-occurring allelic variants of the PRO172, PRO175, PRO178, PRO188, PRO356, PRO179, PRO197, PRO198, PRO182, PRO195, PRO200, PRO211, PRO217, PRO219, PRO221, PRO224, PRO228, PRO245, PRO246, PRO258, PRO261, PRO272, PRO301, PRO322, PRO328, PRO331, PRO364, PRO366, PRO535, PRO819, PRO826, PRO1160, PRO1186 or PRO1246 polypeptide. In certain embodiments of the invention, the native-sequence PRO172, PRO175, PRO178, PRO188, PRO356, PRO179, PRO197, PRO198, PRO182, PRO195, PRO200, PRO211, PRO217, PRO219, PRO221, PRO224, PRO228, PRO245, PRO246, PRO258, PRO261, PRO272, PRO301, PRO322, PRO328, PRO331, PRO364, PRO366, PRO535, PRO819, PRO826, PRO1160, PRO1186 or PRO1246 polypeptide is a mature or full-length native sequence PRO172, PRO175, PRO178, PRO188, PRO356, PRO179, PRO197, PRO198, PRO182, PRO195, PRO200, PRO211, PRO217, PRO219, PRO221, PRO224, PRO228, PRO245, PRO246, PRO258, PRO261, PRO272, PRO301, PRO322, PRO328, PRO331, PRO364, PRO366, PRO535, PRO819, PRO826, PRO1160, PRO1186 or PRO1246 polypeptide comprising the amino acid sequence of Figure 2 (SEQ ID NO:2), Figure 4 (SEQ ID NO:9), Figure 6 (SEQ ID NO:11), Figure 8 (SEQ ID NO:16), Figure 10 (SEQ ID NO:21), Figure 12 (SEQ ID NO:26), Figure 14 (SEQ ID NO:31), Figure 16 (SEQ ID NO:36), Figure 18 (SEQ ID NO:41), Figure 20 (SEQ ID NO:46), Figure 22

(SEQ ID NO:51), Figure 24 (SEQ ID NO:57), Figure 26 (SEQ ID NO:62), Figure 28 (SEQ ID NO:67), Figure 30 (SEQ ID NO:72), Figure 32 (SEQ ID NO:77), Figure 34 (SEQ ID NO:82), Figure 36 (SEQ ID NO:91), Figure 38 (SEQ ID NO:96), Figure 40 (SEQ ID NO:101), Figure 42 (SEQ ID NO:108), Figure 44 (SEQ ID NO:113), Figure 46 (SEQ ID NO:119), Figure 48 (SEQ ID NO:127), Figure 50 (SEQ ID NO:132), Figure 52 (SEQ ID NO:137), Figure 54 (SEQ ID NO:142), Figure 56 (SEQ ID NO:152), Figure 58 (SEQ ID NO:154), Figure 60 (SEQ ID NO:156), Figure 62 (SEQ ID NO:158), Figure 64 (SEQ ID NO:160), Figure 66 (SEQ ID NO:165) or Figure 68 (SEQ ID NO:167), respectively. Fragments of the respective native polypeptides herein include, but are not limited, to polypeptide variants from which the native N-terminal signal sequence has been fully or partially deleted or replaced by another sequence, and extracellular domains of the respective native sequences, regardless whether such truncated (secreted) forms occur in nature. Fragments are preferably sufficient in length for the production of an antibody specifically binding the corresponding native "PRO" polypeptide.

"PRO172 variant polypeptide" means an active PRO172 polypeptide (other than a native sequence PRO172 polypeptide) as defined below having at least about 80% amino acid sequence identity with the amino acid sequence of (a) residues 1 or about 20 to 723 of the PRO172 polypeptide shown in Figure 2 (SEQ ID NO:2), (b) X to 723 of the PRO172 polypeptide shown in Figure 2 (SEQ ID NO:2), wherein X is any amino acid residue from 15 to 24 of Figure 2 (SEQ ID NO:2), (c) 1 or about 20 to X of Figure 2 (SEQ ID NO:2), wherein X is any amino acid from amino acid 539 to amino acid 548 of Figure 2 (SEQ ID NO:2) or (d) another specifically derived fragment of the amino acid sequence shown in Figure 2 (SEQ ID NO:2).

"PRO175 variant polypeptide" means an active PRO175 polypeptide (other than a native sequence PRO175 polypeptide) as defined below having at least about 80% amino acid sequence identity with the amino acid sequence of (a) residues 1 or about 26 to 177 of the PRO175 polypeptide shown in Figure 4 (SEQ ID NO:9), (b) X to 177 of the PRO175 polypeptide shown in Figure 4 (SEQ ID NO:9), wherein X is any amino acid residue from 21 to 30 of Figure 4 (SEQ ID NO:9), (c) X to 177 of the PRO175 polypeptide shown in Figure 4 (SEQ ID NO:9), wherein X is any amino acid from amino acid 47 to amino acid 56 of Figure 4 (SEQ ID NO:9) or (d) another specifically derived fragment of the amino acid sequence shown in Figure 4 (SEQ ID NO:9).

"PRO178 variant polypeptide" means an active PRO178 polypeptide (other than a native

sequence PRO819 polypeptide) as defined below having at least about 80% amino acid sequence identity with the amino acid sequence of (a) residues 1 or about 25 to 52 of the PRO819 polypeptide shown in Figure 60 (SEQ ID NO:156), (b) X to 52 of the PRO819 polypeptide shown in Figure 60 (SEQ ID NO:156), wherein X is any amino acid residue from 20 to 29 of Figure 60 (SEQ ID NO:156), or (c) another specifically derived fragment of the amino acid sequence shown in Figure 60 (SEQ ID NO:156).

"PRO826 variant polypeptide" means an active PRO826 polypeptide (other than a native sequence PRO826 polypeptide) as defined below having at least about 80% amino acid sequence identity with the amino acid sequence of (a) residues 1 or about 23 to 99 of the PRO826 polypeptide shown in Figure 62 (SEQ ID NO:158), (b) X to 99 of the PRO826 polypeptide shown in Figure 62 (SEQ ID NO:158), wherein X is any amino acid residue from 18 to 27 of Figure 62 (SEQ ID NO:158), or (c) another specifically derived fragment of the amino acid sequence shown in Figure 62 (SEQ ID NO:158).

"PRO1160 variant polypeptide" means an active PRO1160 polypeptide (other than a native sequence PRO1160 polypeptide) as defined below having at least about 80% amino acid sequence identity with the amino acid sequence of (a) residues 1 or about 20 to 90 of the PRO1160 polypeptide shown in Figure 64 (SEQ ID NO:160), (b) X to 90 of the PRO1160 polypeptide shown in Figure 64 (SEQ ID NO:160), wherein X is any amino acid residue from 15 to 24 of Figure 64 (SEQ ID NO:160), or (c) another specifically derived fragment of the amino acid sequence shown in Figure 64 (SEQ ID NO:160).

"PRO1186 variant polypeptide" means an active PRO1186 polypeptide (other than a native sequence PRO1186 polypeptide) as defined below having at least about 80% amino acid sequence identity with the amino acid sequence of (a) residues 1 or about 20 to 105 of the PRO1186 polypeptide shown in Figure 66 (SEQ ID NO:165), (b) X to 105 of the PRO1186 polypeptide shown in Figure 66 (SEQ ID NO:165), wherein X is any amino acid residue from 15 to 24 of Figure 66 (SEQ ID NO:165), or (c) another specifically derived fragment of the amino acid sequence shown in Figure 66 (SEQ ID NO:165).

"PRO1246 variant polypeptide" means an active PRO1246 polypeptide (other than a native sequence PRO1246 polypeptide) as defined below having at least about 80% amino acid sequence identity with the amino acid sequence of (a) residues 1 or about 16 to 536 of the PRO1246 polypeptide shown in Figure 68 (SEQ ID NO:167), (b) X to 536 of the PRO1246

identity, more preferably at least about 88% amino acid sequence identity, more preferably at least about 89% amino acid sequence identity, more preferably at least about 90% amino acid sequence identity, more preferably at least about 91% amino acid sequence identity, more preferably at least about 92% amino acid sequence identity, more preferably at least about 93% amino acid sequence identity, more preferably at least about 94% amino acid sequence identity, more preferably at least about 95% amino acid sequence identity, more preferably at least about 96% amino acid sequence identity, more preferably at least about 97% amino acid sequence identity, more preferably at least about 98% amino acid sequence identity and yet more preferably at least about 99% amino acid sequence identity with (a) residues 1 or about 20 to 90 of the PRO1160 polypeptide shown in Figure 64 (SEQ ID NO:160), (b) X to 90 of the PRO1160 polypeptide shown in Figure 64 (SEQ ID NO:160), wherein X is any amino acid residue from 15 to 24 of Figure 64 (SEQ ID NO:160), or (c) another specifically derived fragment of the amino acid sequence shown in Figure 64 (SEQ ID NO:160).

Ordinarily, a PRO1186 variant polypeptide will have at least about 80% amino acid sequence identity, more preferably at least about 81% amino acid sequence identity, more preferably at least about 82% amino acid sequence identity, more preferably at least about 83% amino acid sequence identity, more preferably at least about 84% amino acid sequence identity, more preferably at least about 85% amino acid sequence identity, more preferably at least about 86% amino acid sequence identity, more preferably at least about 87% amino acid sequence identity, more preferably at least about 88% amino acid sequence identity, more preferably at least about 89% amino acid sequence identity, more preferably at least about 90% amino acid sequence identity, more preferably at least about 91% amino acid sequence identity, more preferably at least about 92% amino acid sequence identity, more preferably at least about 93% amino acid sequence identity, more preferably at least about 94% amino acid sequence identity, more preferably at least about 95% amino acid sequence identity, more preferably at least about 96% amino acid sequence identity, more preferably at least about 97% amino acid sequence identity, more preferably at least about 98% amino acid sequence identity and yet more preferably at least about 99% amino acid sequence identity with (a) residues 1 or about 20 to 105 of the PRO1186 polypeptide shown in Figure 66 (SEQ ID NO:165), (b) X to 105 of the PRO1186 polypeptide shown in Figure 66 (SEQ ID NO:165), wherein X is any amino acid residue from 15 to 24 of Figure 66 (SEQ ID NO:165), or (c) another specifically derived

PRO261, PRO272, PRO301, PRO322, PRO328, PRO331, PRO364, PRO366, PRO535, PRO819, PRO826, PRO1160, PRO1186 or PRO1246 polypeptide to a cellular receptor. All such points of intervention by a PRO172, PRO175, PRO178, PRO188, PRO356, PRO179, PRO197, PRO198, PRO182, PRO195, PRO200, PRO211, PRO217, PRO219, PRO221, PRO224, PRO228, PRO245, PRO246, PRO258, PRO261, PRO272, PRO301, PRO322, PRO328, PRO331, PRO364, PRO366, PRO535, PRO819, PRO826, PRO1160, PRO1186 or PRO1246 polypeptide antagonist shall be considered equivalent for purposes of this invention. The antagonists inhibit the mitogenic, angiogenic, or other biological activity of PRO172, PRO175, PRO178, PRO188, PRO356, PRO179, PRO197, PRO198, PRO182, PRO195, PRO200, PRO211, PRO217, PRO219, PRO221, PRO224, PRO228, PRO245, PRO246, PRO258, PRO261, PRO272, PRO301, PRO322, PRO328, PRO331, PRO364, PRO366, PRO535, PRO819, PRO826, PRO1160, PRO1186 or PRO1246 polypeptides, and thus are useful for the treatment of diseases or disorders characterized by undesirable excessive neovascularization, including by way of example tumors, and especially solid malignant tumors, rheumatoid arthritis, psoriasis, atherosclerosis, diabetic and other retinopathies, retrolental fibroplasia, age-related macular degeneration, neovascular glaucoma, hemangiomas, thyroid hyperplasias (including Grave's disease), corneal and other tissue transplantation, and chronic inflammation. The antagonists also are useful for the treatment of diseases or disorders characterized by undesirable excessive vascular permeability, such as edema associated with brain tumors, ascites associated with malignancies, Meigs' syndrome, lung inflammation, nephrotic syndrome, pericardial effusion (such as that associated with pericarditis), and pleural effusion. In a similar manner, the term "agonist" is used in the broadest sense and includes any molecule that mimics a biological activity of a native PRO172, PRO175, PRO178, PRO188, PRO356, PRO179, PRO197, PRO198, PRO182, PRO195, PRO200, PRO211, PRO217, PRO219, PRO221, PRO224, PRO228, PRO245, PRO246, PRO258, PRO261, PRO272, PRO301, PRO322, PRO328, PRO331, PRO364, PRO366, PRO535, PRO819, PRO826, PRO1160, PRO1186 or PRO1246 polypeptide disclosed herein. Suitable agonist or antagonist molecules specifically include agonist or antagonist antibodies or antibody fragments, fragments, or amino acid sequence variants of native PRO172, PRO175, PRO178, PRO188, PRO356, PRO179, PRO197, PRO198, PRO182, PRO195, PRO200, PRO211, PRO217, PRO219, PRO221, PRO224, PRO228, PRO245, PRO246, PRO258, PRO261, PRO272, PRO301,

PRO322, PRO328, PRO331, PRO364, PRO366, PRO535, PRO819, PRO826, PRO1160, PRO1186 or PRO1246 polypeptides, peptides, small organic molecules, etc.

A "small molecule" is defined herein to have a molecular weight below about 500 daltons.

The term "PRO172, PRO175, PRO178, PRO188, PRO356, PRO179, PRO197, PRO198, PRO182, PRO195, PRO200, PRO211, PRO217, PRO219, PRO221, PRO224, PRO228, PRO245, PRO246, PRO258, PRO261, PRO272, PRO301, PRO322, PRO328, PRO331, PRO364, PRO366, PRO535, PRO819, PRO826, PRO1160, PRO1186 or PRO1246 polypeptide receptor" as used herein refers to a cellular receptor for PRO172, PRO175, PRO178, PRO188, PRO356, PRO179, PRO197, PRO198, PRO182, PRO195, PRO200, PRO211, PRO217, PRO219, PRO221, PRO224, PRO228, PRO245, PRO246, PRO258, PRO261, PRO272, PRO301, PRO322, PRO328, PRO331, PRO364, PRO366, PRO535, PRO819, PRO826, PRO1160, PRO1186 or PRO1246 polypeptide, ordinarily a cell-surface receptor found on vascular endothelial cells, as well as variants thereof that retain the ability to bind PRO172, PRO175, PRO178, PRO188, PRO356, PRO179, PRO197, PRO198, PRO182, PRO195, PRO200, PRO211, PRO217, PRO219, PRO221, PRO224, PRO228, PRO245, PRO246, PRO258, PRO261, PRO272, PRO301, PRO322, PRO328, PRO331, PRO364, PRO366, PRO535, PRO819, PRO826, PRO1160, PRO1186 or PRO1246 polypeptide.

"Antibodies" (Abs) and "immunoglobulins" (Igs) are glycoproteins having the same structural characteristics. While antibodies exhibit binding specificity to a specific antigen, immunoglobulins include both antibodies and other antibody-like molecules that lack antigen specificity. Polypeptides of the latter kind are, for example, produced at low levels by the lymph system and at increased levels by myelomas. The term "antibody" is used in the broadest sense and specifically covers, without limitation, intact monoclonal antibodies, polyclonal antibodies, multispecific antibodies (*e.g.*, bispecific antibodies) formed from at least two intact antibodies, and antibody fragments, so long as they exhibit the desired biological activity.

"Native antibodies" and "native immunoglobulins" are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies among the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (V_H) followed by a number

PRO328, PRO331, PRO364, PRO366, PRO535, PRO819, PRO826, PRO1160, PRO1186 or PRO1246 DNA and encoding a specific antibody epitope. General techniques for generating antibodies, and special protocols for *in situ* hybridization are provided hereinbelow.

iii. Antibody Binding Studies

The results of the cardiovascular, endothelial, and angiogenic study can be further verified by antibody binding studies, in which the ability of anti-PRO172, anti-PRO175, anti-PRO178, anti-PRO188, anti-PRO356, anti-PRO179, anti-PRO197, anti-PRO198, anti-PRO182, anti-PRO195, anti-PRO200, anti-PRO211, anti-PRO217, anti-PRO219, anti-PRO221, anti-PRO224, anti-PRO228, anti-PRO245, anti-PRO246, anti-PRO258, anti-PRO261, anti-PRO272, anti-PRO301, anti-PRO322, anti-PRO328, anti-PRO331, anti-PRO364, anti-PRO366, anti-PRO535, anti-PRO819, anti-PRO826, anti-PRO1160, anti-PRO1186 or anti-PRO1246 antibodies to inhibit the effect of the PRO172, PRO175, PRO178, PRO188, PRO356, PRO179, PRO197, PRO198, PRO182, PRO195, PRO200, PRO211, PRO217, PRO219, PRO221, PRO224, PRO228, PRO245, PRO246, PRO258, PRO261, PRO272, PRO301, PRO322, PRO328, PRO331, PRO364, PRO366, PRO535, PRO819, PRO826, PRO1160, PRO1186 or PRO1246 polypeptides on endothelial cells or other cells used in the cardiovascular, endothelial, and angiogenic assays is tested. Exemplary antibodies include polyclonal, monoclonal, humanized, bispecific, and heteroconjugate antibodies, the preparation of which will be described hereinbelow.

Antibody binding studies may be carried out in any known assay method, such as competitive binding assays, direct and indirect sandwich assays, and immunoprecipitation assays. Zola, Monoclonal Antibodies: A Manual of Techniques (CRC Press, Inc., 1987), pp.147-158.

Competitive binding assays rely on the ability of a labeled standard to compete with the test sample analyte for binding with a limited amount of antibody. The amount of target protein in the test sample is inversely proportional to the amount of standard that becomes bound to the antibodies. To facilitate determining the amount of standard that becomes bound, the antibodies preferably are insolubilized before or after the competition, so that the standard and analyte that are bound to the antibodies may conveniently be separated from the standard and analyte that remain unbound.

Sandwich assays involve the use of two antibodies, each capable of binding to a different immunogenic portion, or epitope, of the protein to be detected. In a sandwich assay, the test

C. Antibodies

Some of the most promising drug candidates according to the present invention are antibodies and antibody fragments that may inhibit the production or the gene product of the genes identified herein and/or reduce the activity of the gene products.

i. Polyclonal Antibodies

Methods of preparing polyclonal antibodies are known to the skilled artisan. Polyclonal antibodies can be raised in a mammal, for example, by one or more injections of an immunizing agent and, if desired, an adjuvant. Typically, the immunizing agent and/or adjuvant will be injected in the mammal by multiple subcutaneous or intraperitoneal injections. The immunizing agent may include the PRO172, PRO175, PRO178, PRO188, PRO356, PRO179, PRO197, PRO198, PRO182, PRO195, PRO200, PRO211, PRO217, PRO219, PRO221, PRO224, PRO228, PRO245, PRO246, PRO258, PRO261, PRO272, PRO301, PRO322, PRO328, PRO331, PRO364, PRO366, PRO535, PRO819, PRO826, PRO1160, PRO1186 or PRO1246 polypeptide or a fusion protein thereof. It may be useful to conjugate the immunizing agent to a protein known to be immunogenic in the mammal being immunized. Examples of such immunogenic proteins include, but are not limited to, keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, and soybean trypsin inhibitor. Examples of adjuvants that may be employed include Freund's complete adjuvant and MPL-TDM adjuvant (monophosphoryl Lipid A or synthetic trehalose dicorynomycolate). The immunization protocol may be selected by one skilled in the art without undue experimentation.

ii. Monoclonal Antibodies

The anti-PRO172, anti-PRO175, anti-PRO178, anti-PRO188, anti-PRO356, anti-PRO179, anti-PRO197, anti-PRO198, anti-PRO182, anti-PRO195, anti-PRO200, anti-PRO211, anti-PRO217, anti-PRO219, anti-PRO221, anti-PRO224, anti-PRO228, anti-PRO245, anti-PRO246, anti-PRO258, anti-PRO261, anti-PRO272, anti-PRO301, anti-PRO322, anti-PRO328, anti-PRO331, anti-PRO364, anti-PRO366, anti-PRO535, anti-PRO819, anti-PRO826, anti-PRO1160, anti-PRO1186 or anti-PRO1246 antibodies may, alternatively, be monoclonal antibodies. Monoclonal antibodies may be prepared using hybridoma methods, such as those described by Kohler and Milstein, Nature, 256:495 (1975). In a hybridoma method, a mouse, hamster, or

other appropriate host animal is typically immunized with an immunizing agent to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the immunizing agent. Alternatively, the lymphocytes may be immunized *in vitro*.

The immunizing agent will typically include the PRO172, PRO175, PRO178, PRO188, PRO356, PRO179, PRO197, PRO198, PRO182, PRO195, PRO200, PRO211, PRO217, PRO219, PRO221, PRO224, PRO228, PRO245, PRO246, PRO258, PRO261, PRO272, PRO301, PRO322, PRO328, PRO331, PRO364, PRO366, PRO535, PRO819, PRO826, PRO1160, PRO1186 or PRO1246 polypeptide or a fusion protein thereof. Generally, either peripheral blood lymphocytes ("PBLs") are used if cells of human origin are desired, or spleen cells or lymph node cells are used if non-human mammalian sources are desired. The lymphocytes are then fused with an immortalized cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell. Goding, Monoclonal Antibodies: Principles and Practice (New York: Academic Press, 1986), pp. 59-103. Immortalized cell lines are usually transformed mammalian cells, particularly myeloma cells of rodent, bovine, and human origin. Usually, rat or mouse myeloma cell lines are employed. The hybridoma cells may be cultured in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, immortalized cells. For example, if the parental cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine ("HAT medium"), which substances prevent the growth of HGPRT-deficient cells.

Preferred immortalized cell lines are those that fuse efficiently, support stable high-level expression of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. More preferred immortalized cell lines are murine myeloma lines, which can be obtained, for instance, from the Salk Institute Cell Distribution Center, San Diego, California and the American Type Culture Collection, Manassas, Virginia. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies. Kozbor, J. Immunol., 133:3001 (1984); Brodeur *et al.*, Monoclonal Antibody Production Techniques and Applications (Marcel Dekker, Inc.: New York, 1987) pp. 51-63.

The culture medium in which the hybridoma cells are cultured can then be assayed for the presence of monoclonal antibodies directed against PRO172, PRO175, PRO178, PRO188,

PRO356, PRO179, PRO197, PRO198, PRO182, PRO195, PRO200, PRO211, PRO217, PRO219, PRO221, PRO224, PRO228, PRO245, PRO246, PRO258, PRO261, PRO272, PRO301, PRO322, PRO328, PRO331, PRO364, PRO366, PRO535, PRO819, PRO826, PRO1160, PRO1186 or PRO1246. Preferably, the binding specificity of monoclonal antibodies produced by the hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA). Such techniques and assays are known in the art. The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson and Pollard, Anal. Biochem., 107:220 (1980).

After the desired hybridoma cells are identified, the clones may be subcloned by limiting dilution procedures and grown by standard methods. Goding, *supra*. Suitable culture media for this purpose include, for example, Dulbecco's Modified Eagle's Medium and RPMI-1640 medium. Alternatively, the hybridoma cells may be grown *in vivo* as ascites in a mammal.

The monoclonal antibodies secreted by the subclones may be isolated or purified from the culture medium or ascites fluid by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

The monoclonal antibodies may also be made by recombinant DNA methods, such as those described in U.S. Patent No. 4,816,567. DNA encoding the monoclonal antibodies of the invention can be readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells of the invention serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. The DNA also may be modified, for example, by substituting the coding sequence for human heavy- and light-chain constant domains in place of the homologous murine sequences (U.S. Patent No. 4,816,567; Morrison *et al.*, *supra*) or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. Such a non-immunoglobulin polypeptide can be substituted for the constant domains of an antibody of the invention, or can be substituted for

the variable domains of one antigen-combining site of an antibody of the invention to create a chimeric bivalent antibody.

The antibodies may be monovalent antibodies. Methods for preparing monovalent antibodies are well known in the art. For example, one method involves recombinant expression of immunoglobulin light chain and modified heavy chain. The heavy chain is truncated generally at any point in the Fc region so as to prevent heavy-chain crosslinking. Alternatively, the relevant cysteine residues are substituted with another amino acid residue or are deleted so as to prevent crosslinking.

In vitro methods are also suitable for preparing monovalent antibodies. Digestion of antibodies to produce fragments thereof, particularly Fab fragments, can be accomplished using routine techniques known in the art.

iii. Human and Humanized Antibodies

The anti-PRO172, anti-PRO175, anti-PRO178, anti-PRO188, anti-PRO356, anti-PRO179, anti-PRO197, anti-PRO198, anti-PRO182, anti-PRO195, anti-PRO200, anti-PRO211, anti-PRO217, anti-PRO219, anti-PRO221, anti-PRO224, anti-PRO228, anti-PRO245, anti-PRO246, anti-PRO258, anti-PRO261, anti-PRO272, anti-PRO301, anti-PRO322, anti-PRO328, anti-PRO331, anti-PRO364, anti-PRO366, anti-PRO535, anti-PRO819, anti-PRO826, anti-PRO1160, anti-PRO1186 or anti-PRO1246 antibodies may further comprise humanized antibodies or human antibodies. Humanized forms of non-human (*e.g.*, murine) antibodies are chimeric immunoglobulins, immunoglobulin chains, or fragments thereof (such as Fv, Fab, Fab', $F(ab')_2$ or other antigen-binding subsequences of antibodies) that contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies include human immunoglobulins (recipient antibody) in which residues from a CDR of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat, or rabbit having the desired specificity, affinity, and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies may also comprise residues that are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin, and all or substantially all of the

FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody preferably also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. Jones *et al.*, Nature, 321: 522-525 (1986); Riechmann *et al.*, Nature, 332: 323-329 (1988); Presta, Curr. Op. Struct. Biol., 2:593-596 (1992).

Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source that is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers (Jones *et al.*, Nature, 321: 522-525 (1986); Riechmann *et al.*, Nature, 332: 323-327 (1988); Verhoeyen *et al.*, Science, 239: 1534-1536 (1988)). by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Patent No. 4,816.567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

Human antibodies can also be produced using various techniques known in the art, including phage display libraries. Hoogenboom and Winter, J. Mol. Biol., 227: 381 (1991); Marks *et al.*, J. Mol. Biol., 222: 581 (1991). The techniques of Cole *et al.* and Boerner *et al.* are also available for the preparation of human monoclonal antibodies. Cole *et al.*, Monoclonal Antibodies and Cancer Therapy, Alan R. Liss. p. 77 (1985) and Boerner *et al.*, J. Immunol., 147(1): 86-95 (1991). Similarly, human antibodies can be made by introducing human immunoglobulin loci into transgenic animals, *e.g.*, mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed that closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; and 5,661,016, and in the following scientific publications: Marks *et al.*, Bio/Technology, 10: 779-783 (1992); Lonberg *et al.*, Nature, 368: 856-859 (1994); Morrison, Nature, 368: 812-813 (1994); Fishwild *et al.*, Nature Biotechnology, 14: 845-851 (1996); Neuberger, Nature Biotechnology, 14: 826 (1996); Lonberg and Huszar, Intern. Rev. Immunol., 13: 65-93 (1995).

iv. Bispecific Antibodies

Bispecific antibodies are monoclonal, preferably human or humanized, antibodies that have binding specificities for at least two different antigens. In the present case, one of the binding specificities is for the PRO172, PRO175, PRO178, PRO188, PRO356, PRO179, PRO197, PRO198, PRO182, PRO195, PRO200, PRO211, PRO217, PRO219, PRO221, PRO224, PRO228, PRO245, PRO246, PRO258, PRO261, PRO272, PRO301, PRO322, PRO328, PRO331, PRO364, PRO366, PRO535, PRO819, PRO826, PRO1160, PRO1186 or PRO1246 polypeptide, the other one is for any other antigen, and preferably for a cell-surface protein or receptor or receptor subunit.

Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy-chain/light-chain pairs, where the two heavy chains have different specificities. Milstein and Cuello, Nature, 305: 537-539 (1983). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of ten different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule is usually accomplished by affinity chromatography steps. Similar procedures are disclosed in WO 93/08829, published 13 May 1993, and in Traunecker *et al.*, EMBO J., 10: 3655-3659 (1991).

Antibody variable domains with the desired binding specificities (antibody-antigen combining sites) can be fused to immunoglobulin constant- domain sequences. The fusion preferably is with an immunoglobulin heavy-chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant region (CH1) containing the site necessary for light-chain binding present in at least one of the fusions. DNAs encoding the immunoglobulin heavy-chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. For further details of generating bispecific antibodies, see, for example, Suresh *et al.*, Methods in Enzymology, 121: 210 (1986).

v. Heteroconjugate Antibodies

Heteroconjugate antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been proposed to target immune-system cells to unwanted cells

(U.S. Patent No. 4,676,980), and for treatment of HIV infection. WO 91/00360; WO 92/200373; EP 03089. It is contemplated that the antibodies may be prepared *in vitro* using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins may be constructed using a disulfide-exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate and those disclosed, for example, in U.S. Patent No. 4,676,980.

vi. Effector Function Engineering

It may be desirable to modify the antibody of the invention with respect to effector function, so as to enhance, *e.g.*, the effectiveness of the antibody in treating cancer. For example, cysteine residue(s) may be introduced into the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated may have improved internalization capability and/or increased complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC). See Caron *et al.*, J. Exp Med., 176: 1191-1195 (1992) and Shopes, J. Immunol., 148: 2918-2922 (1992). Homodimeric antibodies with enhanced anti-tumor activity may also be prepared using heterobifunctional cross-linkers as described in Wolff *et al.* Cancer Research, 53: 2560-2565 (1993). Alternatively, an antibody can be engineered that has dual Fc regions and may thereby have enhanced complement lysis and ADCC capabilities. See Stevenson *et al.*, Anti-Cancer Drug Design, 3: 219-230 (1989).

vii. Immunoconjugates

The invention also pertains to immunoconjugates comprising an antibody conjugated to a cytotoxic agent such as a chemotherapeutic agent, toxin (*e.g.*, an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive isotope (*i.e.*, a radioconjugate).

Chemotherapeutic agents useful in the generation of such immunoconjugates have been described above. Enzymatically active toxins and fragments thereof that can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin,

mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes. A variety of radionuclides are available for the production of radioconjugated antibodies. Examples include ^{212}Bi, ^{131}I, ^{131}In, ^{90}Y, and ^{186}Re.

Conjugates of the antibody and cytotoxic agent are made using a variety of bifunctional protein-coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutareldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta *et al.*, Science, 238: 1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026.

In another embodiment, the antibody may be conjugated to a "receptor" (such streptavidin) for utilization in tumor pretargeting wherein the antibody-receptor conjugate is administered to the patient, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a "ligand" (*e.g.*, avidin) that is conjugated to a cytotoxic agent (*e.g.*, a radionucleotide).

viii. Immunoliposomes

The antibodies disclosed herein may also be formulated as immunoliposomes. Liposomes containing the antibody are prepared by methods known in the art, such as described in Epstein *et al.*, Proc. Natl. Acad. Sci. USA, 82: 3688 (1985); Hwang *et al.*, Proc. Natl Acad. Sci. USA, 77: 4030 (1980); and U.S. Pat. Nos. 4,485,045 and 4,544,545. Liposomes with enhanced circulation time are disclosed in U.S. Patent No. 5,013,556.

Particularly useful liposomes can be generated by the reverse-phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol, and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. Fab' fragments of the antibody of the present invention can be conjugated to the liposomes as described in Martin *et al.*, J. Biol. Chem., 257:

Analysis of the full-length PRO1186 sequence shown in Figure 66 (SEQ ID NO:165) evidences the presence of an important polypeptide domain as shown in Figure 66, wherein the location given for that important polypeptide domain is approximate as described above. Analysis of the full-length PRO1186 sequence evidences the presence of a signal peptide from about amino acid 1 to about amino acid 19. Clone DNA60621-1516 was deposited with the ATCC on August 4, 1998, and is assigned ATCC deposit no. 203091.

An analysis of the Dayhoff database (version 35.45 SwissProt 35), using the ALIGN-2 sequence alignment analysis of the full-length sequence shown in Figure 66 (SEQ ID NO:165), evidenced sequence identity between the PRO1186 amino acid sequence and the following Dayhoff sequences: VPRA_DENPO, LFE4_CHICK, AF034208_1, AF030433_1, A55035, COL_RABIT, CELB0507_9, S67826_1, S34665 and CRU73817_1.

EXAMPLE 37

Isolation of cDNA clones encoding PRO1246

Use of the signal sequence algorithm described in Example 3 above allowed identification of an EST cluster sequence from the Incyte database, designated Incyte EST cluster sequence no. 56853. This Incyte EST cluster sequence no. 56853 was then compared to a variety of expressed sequence tag (EST) databases which included public EST databases (*e.g.*, GenBank) and a proprietary EST DNA database (LIFESEQ®, Incyte Pharmaceuticals, Palo Alto, CA) to identify existing homologies. The homology search was performed using the computer program BLAST or BLAST2 (Altshul *et al.*, Methods in Enzymology, 266:460-480 (1996)). Those comparisons resulting in a BLAST score of 70 (or in some cases, 90) or greater that did not encode known proteins were clustered and assembled into a consensus DNA sequence with the program "phrap" (Phil Green, University of Washington, Seattle, Washington). The consensus sequence obtained therefrom is herein designated DNA56021.

In light of an observed sequence homology between the DNA56021 consensus sequence and an EST sequence encompassed within clone no. 2481345, from the Incyte database, clone no. 2481345 was purchased and the cDNA insert was obtained and sequenced. It was found herein that that insert encoded a full-length protein. The sequence of this cDNA insert is shown in Figures 67A-B (SEQ ID NO:166) and is herein designated DNA64885-1529.

Clone DNA64885-1529 contains a single open reading frame with an apparent translational

initiation site at nucleotide positions 119-121 and ending at the stop codon at nucleotide positions 1727-1729 (Figures 67A-B). The predicted polypeptide precursor is 536 amino acids long (Figure 68; SEQ ID NO:167). The full-length PRO1246 protein shown in Figure 68 has an estimated molecular weight of about 61,450 daltons and a pI of about 9.17.

Analysis of the full-length PRO1246 sequence shown in Figure 68 (SEQ ID NO:167) evidences the presence of a variety of important polypeptide domains as shown in Figure 68, wherein the locations given for those important polypeptide domains are approximate as described above. Analysis of the full-length PRO1246 sequence evidences the presence of the following features: a signal peptide from amino acid 1 to about amino acid 15, a transmembrane domain from about amino acid 347 to about amino acid 365, potential N-glycosylation sites from about amino acid 108 to about amino acid 111, from about amino acid 166 to about amino acid 169, from about amino acid 193 to about amino acid 196, from about amino acid 262 to about amino acid 265, from about amino acid 375 to about amino acid 378, from about amino acid 413 to about amino acid 416, and from about amino acid 498 to about amino acid 501, and amino acid sequence blocks having homology to sulfatase proteins from about amino acid 286 to about amino acid 315, from about amino acid 359 to about amino acid 369 and from amino acid 78 to about amino acid 97. Clone DNA64885-1529 was deposited with the ATCC on November 3, 1998, and is assigned ATCC deposit no. 203457.

An analysis of the Dayhoff database (version 35.45 SwissProt 35), using the ALIGN-2 sequence alignment analysis of the full-length sequence shown in Figure 68 (SEQ ID NO:167) evidenced sequence identity between the PRO1246 amino acid sequence and the following Dayhoff sequences: P_R51355, CELK09C4_1, BCU44852_1, IDS_HUMAN, G65169, E64903, ARSA_HUMAN, GL6S_HUMAN, HSARSF_1 and GEN12648.

EXAMPLE 38

Stimulation of Endothelial Cell Proliferation

This assay is designed to determine whether PRO1186 shows the ability to stimulate adrenal cortical capillary endothelial cell (ACE) growth.

Bovine adrenal cortical capillary endothelial cells (ACE) (from primary culture, maximum of 12-14 passages) were plated in 96-well plates at 500 cells/well per 100 microliter. Assay media included low glucose DMEM, 10% calf serum, 2 mM glutamine, and 1X

penicillin/streptomycin/fungizone. Control wells included the following: (1) no ACE cells added; (2) ACE cells alone; (3) ACE cells plus VEGF (5 ng/ml); and (4) ACE cells plus FGF (5ng/ml). The control or test sample, (in 100 microliter volumes), was then added to the wells (at dilutions of 1%, 0.1% and 0.01%, respectively). The cell cultures were incubated for 6-7 days at 37°C/5% CO_2. After the incubation, the media in the wells was aspirated, and the cells were washed 1X with PBS. An acid phosphatase reaction mixture (100 microliter: 0.1M sodium acetate, pH 5.5, 0.1% Triton X-100, 10 mM p-nitrophenyl phosphate) was then added to each well. After a 2 hour incubation at 37°C, the reaction was stopped by addition of 10 microliters 1N NaOH. Optical density (OD) was measured on a microplate reader at 405 nm.

The activity of PRO1186 was calculated as the fold increase in proliferation (as determined by the acid phosphatase activity, OD 405 nm) relative to (1) cell only background, and (2) relative to maximum stimulation by VEGF. VEGF (at 3-10 ng/ml) and FGF (at 1-5 ng/ml) were employed as an activity reference for maximum stimulation. Results of the assay were considered "positive" if the observed stimulation was ≥ 50% increase over background.

PRO1186 assayed "positive" as follows:

1% dilution = 1.75 fold stimulation

0.1% dilution = 1.39 fold stimulation

0.01% dilution = 1.28 fold stimulation

Compared to VEGF (5 ng/ml) control:

1% dilution = 1.24 fold stimulation

Compared to FGB (5 ng/ml) control:

1% dilution = 1.46 fold stimulation

EXAMPLE 39

Inhibition of Vascular Endothelial Growth Factor (VEGF) Stimulated Proliferation of Endothelial Cell Growth

The ability of various PRO polypeptides to inhibit VEGF stimulated proliferation of endothelial cells was tested. Specifically, bovine adrenal cortical capillary endothelial cells (ACE) (from primary culture. maximum of 12-14 passages) were plated in 96-well plates at 500 cells/well per 100 microliter. Assay media included low glucose DMEM, 10% calf serum, 2 mM glutamine, and 1X penicillin/streptomycin/fungizone. Control wells included the following: (1)